

Applicant : Hara, et al.  
Serial No. : 09/699,133  
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Attorney's Docket No.: 13781-002001 / PH-1074US

### AMENDMENT

Please amend the above-captioned application as follows:

#### In The Claims:

Please cancel claims 1 to 4 and 7, without prejudice.

*Please amend the claims as follows:*

Q1  
5. (Amended) A method for culturing a cell, wherein the method comprises culturing the cell using a carrier for cell culture, wherein the carrier for cell culture comprises a porous membrane and an alginate gel layer which is formed on the porous membrane, wherein the porous membrane is not permeable to alginate gel.

Sub B1  
6. (Amended) A method for piling up a cell, wherein the method comprises:  
(a) forming a cell layer on a carrier, wherein the carrier comprises a porous membrane and an alginate gel layer which is formed on the porous membrane;  
(b) solubilizing an alginate gel layer of the carrier thereby exfoliating the cell layer from a porous membrane of the carrier; and  
(c) piling up the exfoliated cell layer on another cell formed on the carrier.

*Please add the following new claims:*

8. (NEW) The method of claim 5 or claim 6, wherein the alginate gel layer is composed of a calcium alginate gel.

A2  
Sub B1  
9. (NEW) The method of claim 5 or claim 6, wherein the carrier further comprises an extracellular matrix component gel layer or extracellular matrix component (ECM) sponge layer which is formed on the alginate gel layer.

10. (NEW) The method of claim 5 or claim 6, wherein the extracellular matrix component comprises a collagen.

11. (NEW) The method of claim 5, further comprising forming a cell multi-layer.

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12. (NEW) The method of claim 5 or claim 6, wherein the porous membrane comprises a filter, an ultrafiltration membrane, a silicone rubber membrane, a polytetrafluoroethylene resin porous membrane, a nonwoven fabric or a gauze-like mesh.

13. (NEW) The method of claim 5 or claim 6, wherein the porous membrane comprises pores.

14. (NEW) The method of claim 13, wherein the pores are between about 0.02 to 1000  $\mu\text{m}$ .

15. (NEW) The method of claim 9, wherein the extracellular matrix component comprises a collagen, an elastin, a proteoglycan, a glucosaminoglycan, a fibronectin, a laminin, a vitronectin or a heparan sulfate.

16. (NEW) The method of claim 9, wherein the extracellular matrix component comprises a gel comprising collagen type IV, laminin and heparan sulfate.

17. (NEW) The method of claim 5 or claim 6, wherein the thickness of the porous membrane is between about 0.01 to 1 mm, 0.01 to 0.1 mm, or 0.05 to 1 mm.

18. (NEW) The method of claim 5 or claim 6, wherein the thickness of the alginate gel layer is between about 0.1 to 3 mm, 1 to 2 mm, or about 1 mm.

19. (NEW) The method of claim 9, wherein the extracellular matrix component gel layer is between about 0.1 to 1 mm, 0.2 to 0.5 mm, or about 0.4 mm.

20. (NEW) The method of claim 9, wherein the thickness of the extracellular matrix component sponge layer is between about 0.1 to 2 mm, 0.2 to 1 mm, or about 0.5 mm.

21. (NEW) The method of claim 5 or claim 6, wherein the cell is a fibroblast, a vascular endothelial cell, a chondrocyte, a hepatocyte, a small intestine epitheliocyte, an epidermis cornification cell, an osteoblast, a bone marrow mesenchymal cell or a fibroblast.

22. (NEW) The method of claim 5 or claim 6, wherein a cell concentration of between about 10,000 to 15,000 cells/ml is added onto the alginate gel layer.

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22.

22. (NEW) The method of claim 5 or claim 6, further comprising detaching the cells from the porous membrane by solubilizing the alginate gel layer.

24.

23. (NEW) The method of claim 22, wherein solubilization of the alginate gel layer is carried out by use of a chelating agent.

25.

24. (NEW) The method of claim 23, wherein the chelating agent comprises a polyaminocarboxylic acid, an ethylenediaminetetraacetic acid, an ethylene glycol-bis( $\beta$ -aminoethyl ether), an oxycarboxylic acids, or a citric acid.

26.

25. (NEW) A method for making a three-dimensional tissue structure comprising the following steps:

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(a) forming a cell layer on a carrier, wherein the carrier comprises a porous membrane and an alginate gel layer which is formed on the porous membrane;

(b) solubilizing <sup>the</sup> an alginate gel layer of the carrier thereby exfoliating the cell layer from a porous membrane of the carrier; and

(c) piling up the exfoliated cell layer on another cell formed on the carrier, thereby making a three-dimensional tissue structure.

27.

26. (NEW) A method for making a three-dimensional tissue structure comprising culturing a cell using a carrier for cell culture, wherein the carrier for cell culture comprises a porous membrane and an alginate gel layer which is formed on the porous membrane, wherein the porous membrane is not permeable to alginate gel.

28.

27. (NEW) A method for *in vitro* drug permeability testing comprising culturing a cell using a carrier for cell culture, wherein the carrier for cell culture comprises a porous membrane and an alginate gel layer which is formed on the porous membrane, wherein the porous membrane is not permeable to alginate gel.